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VARIATION IN ACOUSTIC OVERSTIMULATION CHANGES TINNITUS CHARACTERISTICS

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Abstract—Tinnitus often occurs after exposure to loud noise. This raises the question of whether repeated exposure to noise increases the risk of developing tinnitus. We thus studied tinnitus development after repeated acoustic overstimulation using startle and auditory brainstem-response techniques applied to Mongolian gerbils. Noise with bandwidths ranging from 0.25 up to 0.5 oct were used for repeated acoustic overstimulation. Auditory brainstem response measurements revealed similar threshold shifts in both groups of up to about 30 dB directly after the acoustic overstimulation. We identified an upper limit in threshold values, which was independent of the baseline values before the noise exposure. Several weeks after the acoustic overstimulation, animals with the noise bandwidth of 0.25 oct showed a permanent threshold shift, while animals of the group with the 0.5-oct noise band featured only a temporary threshold shift. We thus conclude that the threshold shift directly after noise exposure cannot be used as an indicator for the upcoming threshold level several weeks later. By using behavioral measurements, we investigated the frequency-dependent development of tinnitus-related changes in both groups and one group with 1-oct noise bandwidth. The number of animals that show tinnitus-related changes was highest in animals that received noise with the bandwidth 0.5 oct. This number was, in contrast to the number of animals in the 0.25-oct bandwidth, not significantly increased after repeated overstimulation. The frequency distribution of tinnitus-related changes ranged from 4 to 20 kHz. In the group with the narrow-band noise (0.25 oct) changes center at one frequency range from 10 to 12 kHz. In the group with the broader noise band (0.5 oct), however, two peaks at 8–10 kHz and at 16–18 kHz were found, which suggests that different mechanisms underlie the tinnitus development.

Key words: acoustic startle response, PPI, GPIAS, overstimulation, ABR, Mongolian gerbil.

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Abbreviations: ABR, auditory brainstem response; ASR, acoustic startle response; GPIAS, gap-in-noise inhibition of acoustic startle; IC, *inferior colliculus*; IHC, inner hair cells; Oct, octave; PPI, pre-pulse inhibition; SPL, sound pressure level.

INTRODUCTION

Worldwide, millions of people hear sounds without a detectable source, a phenomenon called tinnitus. The phantom noise sensation of a subjective tinnitus occurs without internal or external sound source and it is believed that one of the main causes of subjective chronic tinnitus is damage to the inner ear from acoustic overstimulation or Menière disease (Spoendlin and Schrott, 1987; Davis and Rafaie, 2000). However, tinnitus is not considered to be a causative disease, but rather a syndrome of different disorders presumably localized, both, in the inner ear and in the central auditory pathway (Noreña and Farley, 2013).

Initiation and early causes of tinnitus are mostly related to peripheral damage. Acoustic overstimulation, for example, initially affects different structures in the inner ear. Inner hair cells (IHC) are in the central focus of investigations, given their central role for transmitting the input signal to the brain (e.g., Liberman 1984; Lenarz et al., 1993). It has been observed that increased glutamate release from the IHCs after an acoustic trauma led to a reversible swelling of the dendrites of the cochlear nerve (Spoendlin, 1971; Robertson, 1983). Overstimulation with very loud noise can lead to a permanent shift in the hearing threshold and irreversible damage to hair bundles and destruction of hair cells (Wang et al., 2002; Kujawa and Liberman, 2009), often followed by the degeneration of auditory nerve fibers and loss of spiral ganglion cells (Pujol and Puel, 1999; Kujawa and Liberman, 2009; Lin et al., 2011). Chronic tinnitus, on the other hand, is of central origin, as the phantom noise sensation remains even after transection of the auditory nerve (House and Brackmann, 1981; Wigand et al., 1982). This form of tinnitus is related to brain plasticity responding to the pathological activity patterns following inner ear damage (Okamoto et al., 2010; Engineer et al., 2011).

The mechanisms underlying the development of tinnitus are largely unknown and only some models (e.g., Schaette and Kempter, 2006; Parra and Pearlmutter, 2007; Zeng, 2013) give suggestions how input loss and the development of tinnitus are related. Manifestation of plasticity-related changes during tinnitus development can be found in the dorsal cochlear nucleus (DCN), *inferior colliculus* (IC) and the auditory cortex (AC) (e.g., Eggermont and Komiya, 2000; Valentine et al., 2004; Scholl and Wehr, 2008; Koehler and Shore, 2013; Manzoor et al., 2013; Niu et al., 2013). These changes are accompanied by changes in limbic structures (Kraus

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and Canlon, 2012), which probably relate to the influence of attention and emotions such as stress in tinnitus manifestation (Melcher et al., 2000; Lockwood et al., 2001). The syndrome-like characteristics of tinnitus are especially related to the large variety of phantom percepts between patients (Eggermont, 2012). Tinnitus of mainly tonal appearance can have very different pitches ranging from 0.5 up to 16 kHz (e.g., Noreña et al., 2002; König et al., 2006; Roberts et al., 2008). Non-tonal tinnitus is often described in the categories of noise, whistling, hissing, buzzling and chirping. All these different types of tinnitus cannot be easily related to the cause and circumstances of induction (Nicolas-Puel et al., 2006; Kreuzer et al., 2012), which makes the search for underlying mechanisms rather complicated.

Research using animal models can help to disentangle such mechanisms underlying tinnitus induction and development. The induction of tinnitus through acoustic overstimulation is in this context a very useful model, as tinnitus following noise trauma is in the majority of cases described as "tonal" by tinnitus patients (Kreuzer et al., 2012). This suggests that a rather localized and defined damage in the central auditory pathway is substantially contributing to the pathology. The gerbil model of trauma-induced tinnitus is well compatible with the notion mentioned above. Several weeks after noise trauma the finally developing tinnitus, as characterized at the behavioral level, has a narrow, spectrally well-defined characteristic at or slightly above the frequency range of the inducing noise (Nowotny et al., 2011).

This rather simple relationship is not the common situation in rodent models for tinnitus. Tinnitus in rats and mice was described at different positions and of different spectral width (e.g., Longenecker and Galazyuk, 2011; Turner et al., 2012; Lobarinas et al., 2013; Rüttiger et al., 2013; Singer et al., 2013). While inducing stimuli (pure tone, noise band) were somewhat different in these studies, the relationship with the induced tinnitus was not simple. The only consistency was that in almost all cases the induced tinnitus was at higher frequencies (e.g., Longenecker and Galazyuk, 2011; Dehmel et al., 2012; Turner et al., 2012). In addition, it should be noted that also in the gerbil model the situation regarding the spectral content of the tinnitus sensation is less clear in the first phase of tinnitus development when the startle-based detection of tinnitus finds more broadband and unstable pattern of tinnitus (Nowotny et al., 2011).

In order to determine possibly underlying mechanisms of tinnitus induction it is therefore necessary to investigate the dependence of tinnitus on details of the inducing stimulation. This is the aim of the present study. By systematically controlling the width and repetition pattern of the traumatizing noise we investigate important factors of tinnitus induction. The severity, width and spectral pattern of the resulting percept was investigated and revealed interesting patterns and a systematic relation to the parameters of tinnitus induction.

EXPERIMENTAL PROCEDURES

Animals

Twenty-nine Mongolian gerbils (*Meriones unguiculatus*), obtained from the institute's breeding colony, weighing on average 47 ± 18 g and with starting age between 3 and 5 months were used in these experiments. Gerbils were housed in small groups, males separated from females, in a light–dark cycle of 12:12 h with food and water accessible *ad libitum*. Experimental procedures were approved according to federal regulations (approved by Regierungspraesidium Darmstadt, number F104/53). To compare effects of noise-induced hearing loss and tinnitus development, we measured auditory brain stem responses (ABRs) and startle response-based behavior in the same animals. Since in earlier studies (Nowotny et al., 2011; Serra et al., 2015) no correlations between outer hair cell damage and tinnitus were observed, distortion product otoacoustic emissions (DPOAEs) were not measured.

General anesthesia

Animals were anesthetized for ABR measurements and acoustic overstimulation with a mixture of 100 mg/ml ketamine (Ketavet®, Pfizer), 2% xylazine (Rompun®, Bayer) diluted in physiological saline solution (0.9%, Braun) at a ratio of 4.5:1:4.5. Anesthesia was induced with an initial dose of 0.25 ml per 100 g body weight (Goss-Sampson and Kriss, 1991; Nowotny et al., 2011; Althen et al., 2012). In literature, the use of ketamine/xylazine does not alter ABR amplitudes (Smith and Mills, 1989). When data on ABR threshold shift directly after the noise trauma were compared between the anesthetized and the awake states, no effect of ketamine use was found (e.g., Popelár et al., 1987; Syka et al., 1994). Depth of anesthesia was controlled with the toe-pinch reflex and by checking for movement of the vibrissae. The animal was placed on a heating pad to maintain body temperature at 38 °C and a micropump (WPI, sp100i syringe pump, World Precision Instruments, Sarasota, FL, USA) was used for continuous infusion of anesthetics (0.04 ml/h, i.p.) during the entire experiment.

ABRs and acoustic overstimulation

ABR measurements are used to obtain information about auditory sensitivity at the levels of the auditory nerve up to the IC. Pure-tone-evoked ABR responses can be registered at the skull surface with the help of the far-field technique (Jewett et al., 1970). We used short pure tones (10 ms, 0.5 ms r/f) with stimulation frequencies from 2 to 20 kHz (2 kHz steps) and sound pressure levels from 0 to 80 dB SPL (sometimes 90 dB SPL) in 5-dB steps. The recording experiment including stimulus generation was run by a custom-built computer program written in MATLAB (The MathWorks, Inc.; Version 2007b, Natick, MA, USA). Computer-generated waveforms were transferred to an internal sound card (ESI Juli@24-bit/192 kHz, Leonberg, Germany, in some experiments. DAP 3000A-212). Signals were amplified (Rotel RB 1510,

North Reading, MA, USA) and broadcasted via loudspeaker (MHT 12 8 Ω, Visaton®, Haan, Germany) located 10 cm from the left ear of the animal. ABRs were recorded differentially from two subcutaneous positions using silver electrodes. The (−)-electrode was placed close to the left auditory bulla and the (+)-electrode above the right IC. An additional reference electrode was positioned at the caudal end of the back close to the tail root. For each stimulus, measurements were repeated 400 times with an inter-stimulus interval of 100 ms and averaged to achieve a better signal-to-noise ratio. Signals from the electrodes were sent via preamplifier to a differential amplifier (Cornerstone, Ex1, Dagan Corporation, Minneapolis, Minnesota, USA) with a gain of 2.000 and a band-pass filter set to 0.3–2 kHz. The signal was then fed back into the soundcard (sampling rate 96 kHz) and stored in the computer for further analysis. ABR threshold values were determined through visual inspection and were double-checked by an independent observer. ABR thresholds were determined directly before and again immediately after acoustic overstimulation. As thresholds were always elevated after the noise trauma, the measurement range was adjusted to the range 60 up to 90 dB SPL (5-dB steps). ABR threshold data (SPL was lowest to evoke an ABR response) from all frequency points were combined per animal for individual hearing-threshold curves.

Acoustic overstimulation for tinnitus induction was performed using System 3 hardware and RPVDs software from Tucker Davis Technologies (Alachua, USA). Noise bands around a center frequency of 8 kHz were generated in a signal processor (RP2. 1 processor), attenuated (programmable attenuator PA5) to the desired SPL of 105 dB SPL (1 h, peak-to-peak, which corresponds to 95.3 dB SPL RMS), then amplified (Rotel, RB 1510, North Reading, USA) and presented free-field from 20 cm above the animal via a horn speaker (HTH 8.7, Visaton, Haan, Germany). Three different animal groups were overstimulated with noise bands around 8 kHz of different widths: 0.25-octave wide (narrow, 7.3–8.7 kHz), 0.5-octave wide (medium, 6.7–9.5 kHz), and 1-octave wide (wide, 5.7–11.3 kHz). For the animal groups with 0.25-oct noise ($n = 10$) and with 0.5-oct noise ($n = 11$) the same acoustic overstimulation was repeated eight weeks after the first one. As for the first trauma, again, hearing thresholds were determined by ABR measurements before and after the second overstimulation.

ABR peak amplitude analysis

Response changes along the auditory path were investigated with a peak amplitude analysis of the ABR waveforms. The amplitudes of individual maxima (peaks) were examined at different sound pressure levels (0–80 dB SPL) for each animal and test frequency (custom-built software under MATLAB R2007b, the MathWorks, Inc.; MA, USA). Individual amplitude maxima for the different peaks were averaged across animals for the measurements: before trauma, eight weeks after the first trauma and eight weeks after the second trauma.

Startle response measurements

Characteristics of induced tinnitus perception were detected by acoustic startle response (ASR) behavioral measurements. Thereby, mainly the frequency characteristics could be determined, with its temporal development characterized through repeated measurements. Detailed information about the procedure of ASR measurements in gerbils was published previously (Nowotny et al., 2011). In brief, a measuring platform (Med Associates Inc., St. Albans, VT, USA) was used to sense the response amplitude of the acoustically elicited whole-body startle response, which was induced by a sudden, loud noise burst. The response signal passed a charging amplifier (PHM 250B; Med Associates Inc., St. Albans, USA) and was amplified (4×) and filtered (1 Hz to 3 kHz) before it was sent back into an external soundcard (Fireface 400, RME, Heimhausen, Germany). A custom-built MATLAB program (MATLAB R2007b, The MathWorks, Inc., MA, USA, kindly provided by Manfred Koessl) was used for generation of the stimulus and for analysis of the measured signal.

After a habituation phase of approximately ten minutes at the beginning of each recording session, animals were exposed to eight standard startle-evoking stimuli (BBN: 1.5 kHz to 20 kHz, duration: 20 ms, rise/fall-time: 10 μs at 105 dB SPL (inter-stimulus interval: 14 s), which were not incorporated in the evaluation. For all ASR measurements, each stimulus or stimulus combination was repeated 25 times in a randomized sequence within a stimulus paradigm. The general startle behavior of the individual animals was characterized with an input–output function (I/O) of the ASR. For this, the SPL of the startle stimulus was varied in steps of 10 dB between 65 and 115 dB SPL. The ASR can be inhibited by the presentation of a non-startling pre-pulse occurring shortly before the ASR-eliciting stimulus. This is called pre-pulse inhibition (PPI). The amount of PPI can be seen as an indicator for hearing sensitivity (Yang et al., 2007), which can change after noise overstimulation. PPI measurements were important when attempting to determine tinnitus rather uninfluenced by noise-induced hearing loss. A noise burst pre-pulse (duration: 20 ms, at 75 dB SPL) was presented starting 100 ms before the ASR stimulus. These pulses were presented with ten different center frequencies (2–20 kHz in 2 kHz steps), each with a bandwidth of 0.125 oct.

The so-called gap-induced PPI of the ASR (gap-PPI) for tinnitus detection makes use of the fact that the ASR is also inhibited by a pre-pulse in the form of a gap in background noise right before the startle stimulus (Hoffman and Ison, 1980; Koch, 1999; Gaese et al., 2009). We used a 50-ms gap starting 100 ms before the startle stimulus. This gap pre-pulse was presented in background noise (bandwidth 0.125 oct, at 75 dB SPL) centered at ten different frequencies (2–20 kHz in 2-kHz steps). For each center frequency the paradigm included trials with and without gap pre-pulse for comparison. One stimulus trial had a duration of 14 s with the background noise starting after 5 s, the pre-pulse at 9.4 s, and the startle stimulus starting at 9.5 s.

Absolute startle response amplitudes were determined by subtracting the maximal amplitude of the spontaneous motor activity (100 ms time window before startle stimulus or pre-pulse) from the maximal amplitude of the ASR (100 ms time window after startle stimulus onset). If the spontaneous activity in a given trial was too high and the resulting value was lower than −0.4 V, this measurement was not used for further evaluation. The percentage of PPI was quantified by PPI (%) = (ASR without gap−ASR with gap)/ASR without gap * 100. Using Sigma Plot (Version 10.0, Systat Software, Inc., Erkrath, Germany), the values from all test animals were averaged as mean ± standard deviation (SD) and plotted as a function of SPL (I/O) or center frequency of the background noise (PPI, gap-PPI).

The number of animals showing a significant reduction in gap-PPI was counted by using a threshold criterion for the reduction of >2 SD from the baseline PPI values (Fig. 1). For average baseline SD calculation, we used the measured gap-PPI values from all animals before the noise trauma. To determine the SD that is used as criterion, gap-PPI value at each stimulus frequency were averaged (Fig. 1). For comparing the three different bandwidth groups (0.25–1 oct trauma bandwidth) an average SD of 11.8% (n = 29) was detected, resulting in a threshold criterion of 22% reduction in PPI. For repeated trauma, only two different bandwidth groups (0.25 and 0.5 oct) with an average SD of 12.4% (n = 21) were used, resulting in a threshold criterion of 24% reduction in PPI. This criterion was applied per animal and test frequency.

Statistical analysis

Statistical analyses were performed with JMP (Version 7.0; SAS Institute Inc., Cary, NC, USA). ABR and ASR measurements were analyzed in repeated-measures ANOVAs testing the influences of the parameters frequency (10 values: 2–20 kHz in 2-kHz steps), time point of measurement (five times) and groups. Greenhouse-Geisser-corrected p-values were used for ABR and ASR measurements if necessary (Mauchly's test for sphericity). Post-hoc comparisons were Bonferroni–Holm corrected for multiple comparisons. Few partial analyses were carried out pair-wise using the Wilcoxon's MPSR test (matched-pairs signed rank). Significance was assumed at $p < 0.05$, different levels are marked in the figures as * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).

RESULTS

We determined the risk of tinnitus development after acoustic noise overstimulation in Mongolian gerbils with three different noise bandwidths of 0.25, 0.5 and 1 oct. All groups received a noise trauma of 105 dB SPL and a duration of one hour but with different bandwidths around the center frequency of 8 kHz. At the beginning of the study, ABR recordings and behavioral measurements (ASR) were performed to characterize the normal (healthy) state of the animals. These measurements were repeated after each noise exposure to document hearing threshold shifts (every five weeks thereafter) and tinnitus development (every eight weeks thereafter). Further, over a period of sixteen weeks and two noise traumata in each group, we repeatedly measured noise-induced threshold shifts and tinnitus related changes in the 0.25- and 0.5 oct-bandwidth groups.

Bandwidth of traumatizing noise has a non-linear effect on induced tinnitus

In the first part of the study we investigated the influence of the bandwidth of acoustic overstimulation on the degree and frequency characteristics of tinnitus induction. Using three different bandwidths of 0.25, 0.5 and 1 oct around a center frequency of 8 kHz we determined the reduction in gap-PPI response five

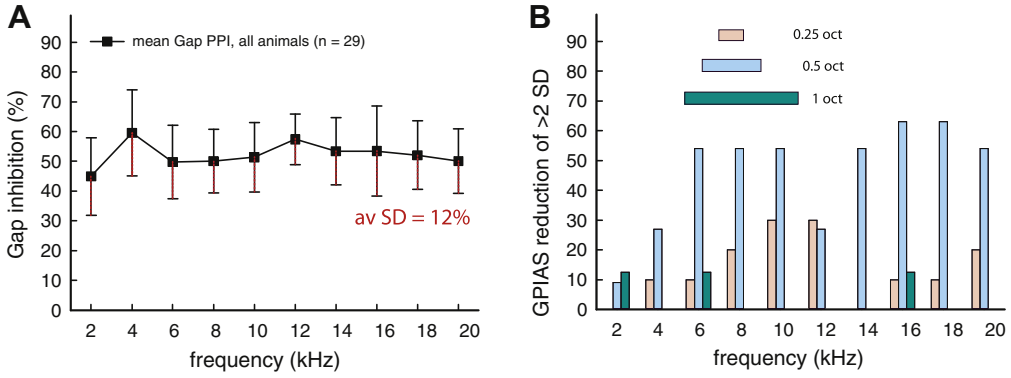

Fig. 1. Frequency distribution of strong reductions in gap-PPI five weeks after acoustic overstimulation. Strong reduction in gap-PPI compared to baseline in individual animals was taken as a clear indication for tinnitus. (A) At each stimulus frequency, the SDs of the baselines were used for threshold determination. Animals of all three groups (n = 29) exhibit an average SD of 12%. Reduction of gap-PPI > 2 × SD (24%) on the individual animal was used as tinnitus-criterion. (B) Percentage of animals with a significant reduction in gap-PPI (criterion as indicated above) is shown as a function of test frequency for the three groups with differences in the noise bandwidth used for overstimulation. The frequency range of the noise bandwidth around 8 kHz used in the three different groups for overstimulation is indicated by horizontal bars above the histogram. GPIAS: gap-in-noise inhibition of acoustic startle.

weeks after the first acoustic overstimulation. As a quantitative measure for the severity of tinnitus induction we counted the number of affected animals with strong reduction in gap-PPI inhibition (>2 SD of baseline gap-PPI values). The strongest reduction in gap-PPI was found for the intermediate bandwidth of 0.5 oct, both in terms of the number of affected test frequencies and in terms of the number of animals affected at a given frequency (Fig. 1). Both, broader traumatizing noise and narrower noise led to a reduction in tinnitus effects.

Further comparisons revealed that trauma induction with narrow-band noise (both, 0.25 oct and 0.5 oct bandwidth) led to a significantly stronger gap-PPI reduction compared to the broad traumatizing noise (1 oct bandwidth), i.e. were related to a higher risk of developing tinnitus (Fig. 1, Wilcoxon MPSR: 0.25–0.5 oct : $n = 8$, $S = 17$, $p = 0.004$, 0.25–1.0 oct: $n = 8$, $S = 13$, $p = 0.03$; 0.5–1.0 oct: $n = 8$, $S = 18$, $p = 0.08$). The 0.5 oct bandwidth led to particularly strong effects: about 63% of the Mongolian gerbils showed strong changes in gap-PPIs at 16 and 18 kHz. To control for hearing impairment, which would interfere with detecting gap-PPI, we additionally measured tone-induced PPI and found no differences in PPI values before and five weeks after acoustic overstimulation (Fig. 7).

In general, the frequency-dependent distribution of tinnitus induction was more extended toward the high-frequency side of the traumatizing noise and was double-peaked. For the medium and narrow traumatizing noise, there was a peak around and slightly above the used trauma frequency band and a second peak in the high-frequency range (14–20 kHz) with a dip in between at around 12 kHz. These two peaks were narrowed down for the broad traumatizing noise to only two test frequencies (4 and 6 kHz; low-frequency range), and to 16 kHz only (high-frequency range). While the non-linear characteristics of the relationship between trauma bandwidth and tinnitus effects, measured five weeks after trauma, were clearly independent of the induced noise trauma, other aspects already became obvious in the trauma-related threshold shifts (see below).

Noise-induced threshold shift directly after the trauma was independent of traumatizing noise bandwidth

In order to determine the effect of an acoustic overstimulation on brainstem responses, we measured ABR thresholds before, immediately and eight weeks after the acoustic overstimulation (Fig. 2). Focusing on the highly effective trauma bandwidths of 0.25 and 0.5 oct, it was obvious that ABR threshold shifts occurred almost entirely at and above trauma frequency (Fig. 2), with shifts up to 30 dB (Fig. 2C). These threshold shifts clearly extended to the high-frequency range above the (highest measured) frequency of 20 kHz while even at the most sensitive threshold values found at 4 kHz (mean 18.7 ± 2.7 dB SPL, $n = 21$) no shift occurred as it was clearly below trauma frequency. A well-comparable pattern of ABR threshold

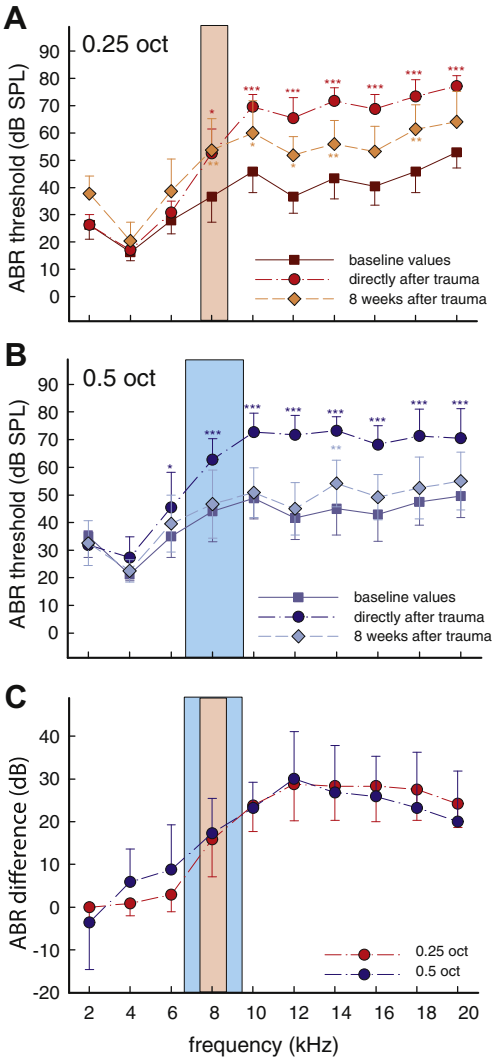

Fig. 2. Auditory brainstem response thresholds measured before (squares), directly after (circles), and eight weeks after (diamonds) the first acoustic trauma. (A) Average ABR thresholds ($n = 10$) before and after noise trauma induced with 0.25-oct noise around 8 kHz (frequency range indicated by red vertical bar). (B) ABR thresholds ($n = 11$) after acoustic overstimulation noise with a bandwidth of 0.5 oct around 8 kHz (indicated by blue vertical bar) performed at frequencies between 2 and 20 kHz in 2-kHz steps. (C) Threshold difference values between baseline and directly after trauma in both groups. Significant differences are indicated as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, values are given as mean ± std. dev.

shifts was induced through the application of overstimulation by different bandwidths, with largest difference between ABR baseline thresholds and those measured directly after trauma at 12 kHz (group 0.25 oct: 28.8 ± 8.6 dB, group 0.5 oct: 30.0 ± 11.1 dB, Fig. 2C).

Long-term threshold recovery was only complete after trauma with 0.5-oct noise

We measured ABR thresholds again eight weeks later to determine threshold recovery. Although threshold shift directly after the acoustic overstimulation were rather

consistent (Fig. 2C), the recovery eight weeks later was clearly different between groups. The narrow-band traumatizing noise (Fig. 2A) led to a permanent threshold shift with still significantly increased threshold values at 8, 10, 12, 14, and 18 kHz after trauma (post hoc: significant differences as indicated in Fig. 2A, black circles). Starting at 8 kHz, the average threshold shift eight weeks after trauma with 0.25-oct noise was 14.3 ± 9.3 dB (n = 10). In contrast, after trauma with 0.5-oct noise a recovery to baseline values could be observed. Only a single significantly increased threshold value remained at 14 kHz (Fig. 2B, black circles). In summary, traumatizing noise bands of 0.25- and 0.5-oct bandwidth around 8 kHz induced well-comparable thresholds shifts in the measured range up to 20 kHz. While thresholds in the 0.5-oct group were well recovered after eight weeks, recovery in the 0.25-oct group remained incomplete. Therefore, threshold shift and recovery were not predictive for the details of tinnitus induction as the trauma effects were stronger in the 0.25-oct group, while the 0.5-oct group had a more prominent pattern of tinnitus induction.

Changes in threshold level across repetitive noise trauma are very consistent

The impact of repeated acoustic overstimulation on ABR threshold was tested by applying a second noise trauma to all animals of both groups eight weeks after the first one. Thresholds in all animals at frequencies below the trauma-band frequencies were at the level of the original baseline values before the second trauma was applied (Fig. 3). At 4 kHz, as for example shown in Fig. 3A, animals in the 0.25-oct group had an average ABR threshold of 20.5 ± 6.9-dB SPL (n = 10). In the 0.5-oct group the threshold at 4 kHz was 21.3 ± 2.3-dB SPL (n = 11; Fig. 3B). The prior conditions for the second trauma were different between both groups in the frequency range above 6 kHz where the first trauma had been effective: while ABR threshold in the 0.25-oct group at frequencies above 6 kHz had remained significantly above baseline values, ABR threshold in the 0.5-oct group had returned to baseline levels. By setting a second noise trauma with the same characteristics as the first one in each group, ABR threshold values increased again significantly (Fig. 3). Well comparable to the first trauma, threshold increase was only obvious in the frequency range of the trauma frequency and above, with resulting levels of on average 68.7-dB SPL (8–20 kHz, n = 21). This was rather comparable in both groups. The maximum threshold shift was again found at 12 kHz, the amount of threshold shift, however, was different depending on the preconditions i.e. bandwidth of traumatizing noise (0.25-oct group: 14.1 ± 0.2 dB; 0.5-oct group: 23.2 ± 7.5 dB; Fig. 4A).

Recovery from the second trauma was again determined eight weeks later. Thresholds had returned to lower values well consistent with the recovery after the first trauma. In detail, this pattern differed between the two groups. The 0.25-oct group kept a less profound but still permanent threshold shift at frequencies above

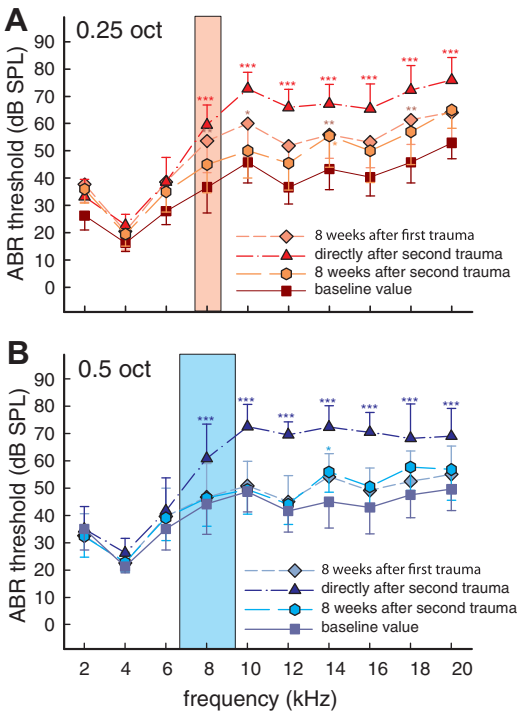

Fig. 3. Trauma-induced ABR threshold shifts in the Mongolian gerbil after repetition of acoustic overstimulation. Mean thresholds measured at different points in time around induction of a second trauma in the two animal groups with different trauma bandwidth: (A) ABR thresholds after overstimulation with noise bandwidth of 0.25 oct (red vertical bar, n = 10) and (B) ABR thresholds after overstimulation with noise bandwidth of 0.5 oct (blue vertical bar, n = 11). Significant differences are indicated as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, values are given as mean ± std. dev.

6 kHz (Fig. 3A, black circles), while in the 0.5-oct group the recovery was almost complete and only one significant difference (at 14 kHz) was found in comparison to the baseline (Fig. 3B, black circles). Recovered thresholds were astonishingly consistent after first and second trauma, especially in the 0.5-oct group (Fig. 3B). Recovery in the 0.25-oct group was, again, not complete (Fig. 3A). For the frequency range around and directly above trauma frequency, the final threshold remained even lower than after the first trauma, while in the frequency range 14 kHz and above the recovered threshold was at the same level as after the first trauma.

The variable influence of a noise trauma on the induced ABR threshold shift and the determining factors were investigated by comparing baseline values and shifts between first and second trauma. A comparison of ABR threshold shift after the second trauma with the baseline values right before any noise application was done (before trauma one), showed that traumatizing noise of different bandwidths led again to comparable long-term threshold shifts that were not significantly different between groups (Fig. 4B). These overall effects were, in addition, well comparable to the threshold shifts induced by the first trauma only (compare to Fig. 2C). Short-term effects can deviate from this general pattern. Due to the difference in baseline values right before the

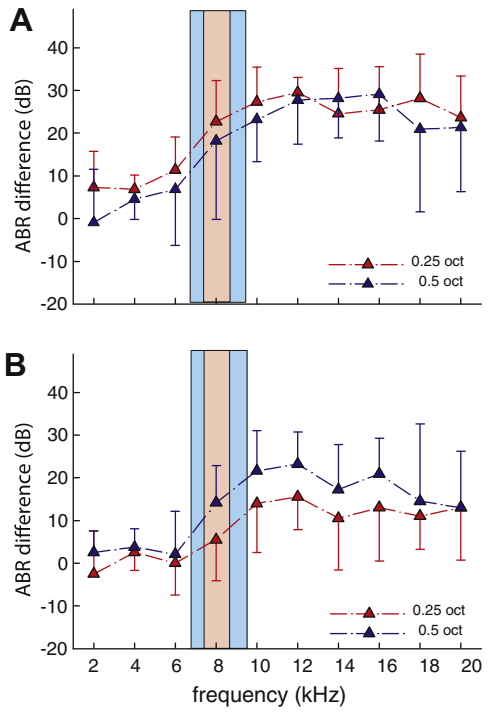

Fig. 4. Noise-induced ABR threshold shifts after repeated acoustic trauma. (A) Threshold shift induced by the second trauma measured as the difference between directly before and directly after the second trauma in both groups. (B) Overall threshold shift resulting from repeated noise trauma (eight weeks between first and second trauma) measured as the difference between baseline levels and threshold directly after the second overstimulation. Values are given as mean ± std. dev.

second trauma, the noise-induced threshold change in the 0.25-oct group was smaller than in the 0.5-oct group (Fig. 4A). Taken together, how strongly ABR thresholds are affected by an acoustic trauma seems to be mainly determined by a limiting upper level for the threshold that can be reached after noise trauma, independent of noise bandwidth and precondition. This assumed limit was on average close to 70 dB SPL (e.g., at 12 kHz, 0.25-oct group, threshold after first trauma: 65.4 ± 7.5 dB SPL, $n = 10$, after second trauma: at 65.9 ± 6.6 dB SPL, $n = 10$).

Decrease of cochlear input to the auditory path is accompanied by an increased central activity only in the 0.5-oct noise group

ABR waveforms can be used to determine hearing thresholds for individual frequencies. We observed changes in ABR waveform amplitudes not only at threshold level but also at higher SPLs (Fig. 5, 80 dB SPL). In both groups, e.g., the amplitude of the first peak in the ABR waveform, which represents the cochlear input (input peak), was reduced eight weeks after the first noise trauma (Fig. 5 left side, auditory nerve activity). A repetition of the noise trauma, however, additionally reduced the ABR amplitude of the input peak only in the 0.5-oct group.

Differences between both trauma groups were also found in later responses (late peak) in the ABR waveforms (Fig. 5 right side, IC activity). While in the 0.25-oct group the amplitude of the late peak decreased eight weeks after the first noise trauma, the amplitude response in the 0.5-oct group was increased. After application of the second noise trauma, the 0.25-oct group and the 0.5-oct group showed no further decrease or increase in amplitude, respectively. A decrease of amplitude values was only documented in the 0.25-oct group at 16 kHz (Fig. 5D, red curve). Concerning the ratio in amplitude between the input and late peak along the auditory path, both groups started with comparable values before the noise trauma (0.25-oct group = 1.1, 0.5-oct group = 1.3). Since both, the input peak and the late peak decreased in amplitude in the 0.25-oct group, the ratio between the peaks was not different (0.25 oct = 1.2). In the 0.5-oct group the amplitude ratio increased to 1.9 due to the opposed development of the input and late peak.

Trauma repetition only increased the risk to develop tinnitus in the 0.25-oct noise group

As induced tinnitus after a first trauma was already strongly affected by noise bandwidth, one would expect even further differentiation after a second trauma. This was quantified by counting the number of animals with strong gap-PPI changes (> 2 SD baseline values) several times after acoustic trauma.

We found that the frequency distribution of tinnitus-related changes was different between both groups. After trauma with 0.25-oct bandwidth, most animals showed deficits in gap-PPI focused in the frequency range around 10 to 12 kHz, which was well above the applied overstimulation (Fig. 6A). In contrast, after trauma with 0.5-oct noise the distribution showed two distinct peaks depending on frequency, one at around 8 kHz and one around 16 kHz (Fig. 6B). The most obvious difference already after the first trauma was that 0.5-oct trauma bandwidth led to a much higher percentage of affected animals. Maximum percentage of affected animals per measured frequency was: 0.25-oct group (at 10 kHz) = 30%; 0.5-oct group (at 16 kHz) = 63%. In both groups, the pattern of tinnitus induction was not strongly affected by the second trauma and the overall distribution remained fairly constant. Although the number of affected animals was higher in the 0.5-oct group, a repeated noise trauma led to a significant increase of affected animals in the 0.25-group only (Fig. 6A, Wilcoxon MPSR: $n = 10$, $S = 19.5$, $p = 0.023$). Comparison at the individual level of each animal, in which we related the gap-PPI changes at the tested frequency five weeks after the first and second noise trauma to each other, supported an increased tinnitus risk in the 0.25-oct group. Most (53.8%) of the counted gap-PPI changes were new gap-PPI reductions after the second trauma. In the 0.5-oct group new changes after the second trauma were only found in 26.1% of the cases and the number of invariable gap-PPI changes after each noise trauma was most dominant (43.5%). Furthermore, an improvement of

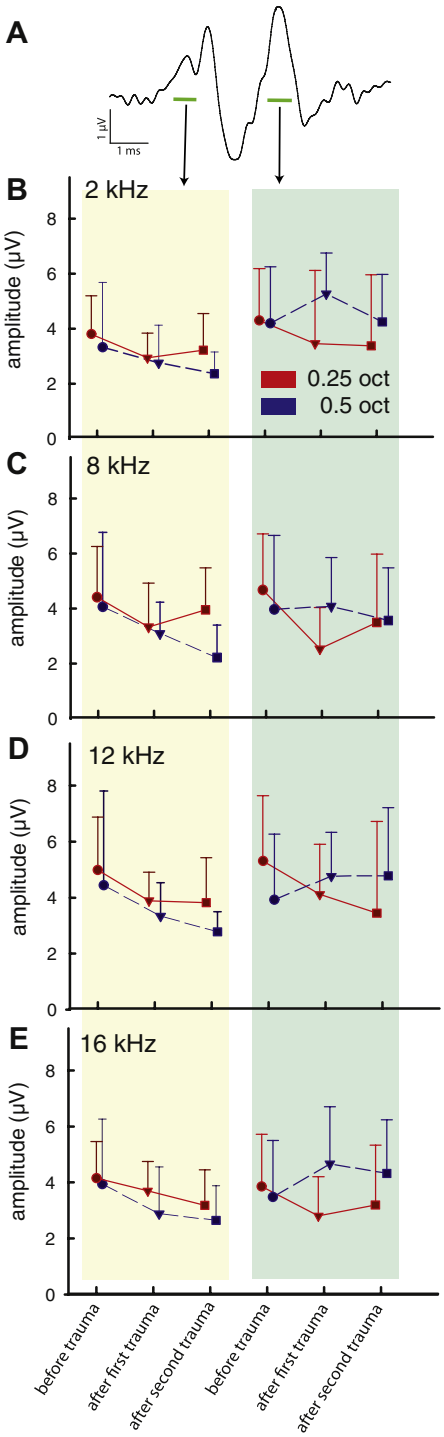

Fig. 5. Average peak amplitude values of the different peaks in the ABR waveforms. (A) Typical example for the average sound-induced ABR wave at 16 kHz from one animal is depicted. The time windows for analysis of the input peak (left) and the late peak (right) are indicated by green bars below the waveform. (B–E) Left side shows the average amplitude of the input peak in the ABR waveform (*nucleus cochlearis* activity) and the right side for the late peak (*inferior colliculus* activity) with values measured before the noise trauma (circles), eight weeks after the first trauma (triangles) and eight weeks after the second trauma (squares). Average amplitude values (0.25 oct: $n = 10$, 0.5 oct: $n = 11$) were determined for 2 kHz (B), 8 kHz (C), 12 kHz (D) and 16 kHz (E).

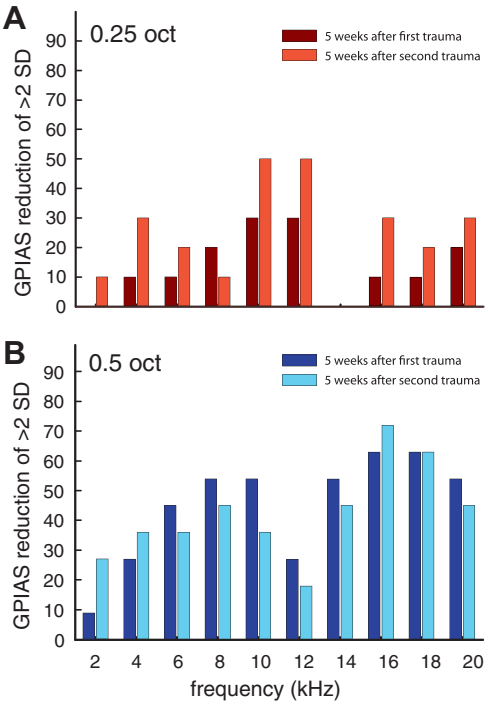

Fig. 6. Frequency distribution of animals indicating tinnitus induction after the first and second trauma. Induced tinnitus was determined as gap-PPI change of > 2 SD (standard deviation) from baseline values as explained in Fig. 1. (A) Percentage of animals with gap-PPI change depending on test frequency in the 0.25-oct group and (B) percentage of animals with gap-PPI change depending on test frequency in the 0.5-oct group. Please note that repetition of noise trauma significantly increased the risk of tinnitus development only in the narrow-bandwidth group (0.25 oct).

animal status (0.25 oct = 3 of 10 animals, 0.5 oct = 7 of 10 animals), by the absence of strong gap-PPI changes at a certain frequency after the second noise trauma, was detected in both groups (percentage of all gap-PPI changes: 0.25 oct = 11.5% of the changes, 0.5 oct = 30.4%).

The observed difference between groups already after the first trauma could have possibly been related to the differentially affected ABR thresholds. While threshold recovery in the 0.5-oct group was complete, this was not the case in the 0.25-oct group. One might argue that this stronger effect in the latter group might have affected general hearing which should lead to incomparable startle measurements. As a control measurement, we tested the influence of 75-dB loud noise pre-pulses on the ASR. Comparable PPI before and after trauma would indicate consistent startle thresholds, which was found for all groups and traumata (Fig. 7). This rules out the possible explanation that the smaller percentage of tinnitus induction is resulting from a general trauma-induced deficit in hearing in this group. The 0.5-oct group, for comparison, showed a non-significant tendency toward reduced noise pulse-PPI after trauma.

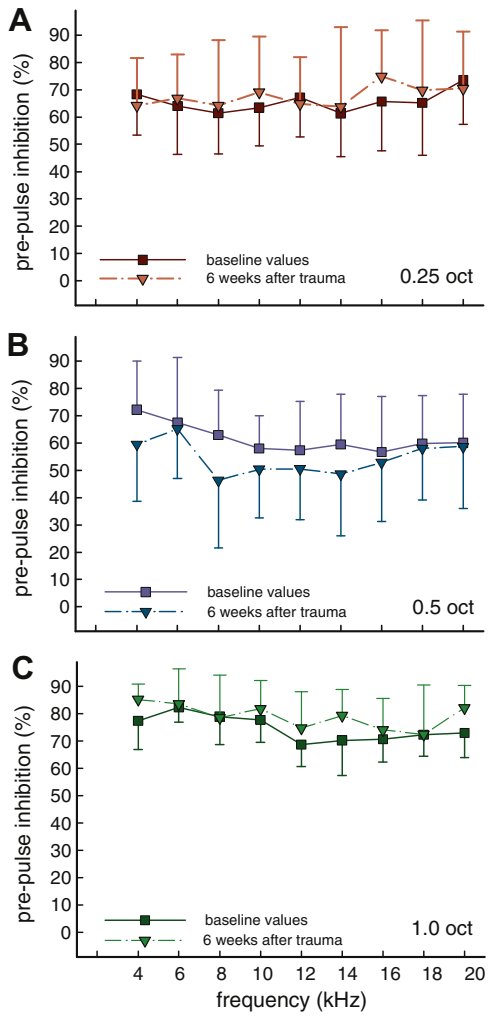

Fig. 7. Pre-pulse inhibition before and after acoustic trauma in all groups with different trauma bandwidths. The average prepulse inhibition is shown as a function of test frequency for the three groups with differences in the frequency width of trauma induction. All groups were tested using noise burst pre-pulses (0.25-oct bandwidth) at ten different test frequencies. Depicted are group data for a trauma bandwidth of (A) 0.25 oct, (B) 0.5 oct, and (C) 1 oct.

DISCUSSION

In the present study, we show that the use of different trauma bandwidths has a nonlinear effect on the risk to develop tinnitus. Especially, noise trauma resulting from medium bandwidths (0.5 oct) bears the highest risk of tinnitus development. However, a repetitive application of a noise trauma only increases the tinnitus risk in the narrow-band noise groups of 0.25 oct. A comparison of the reached hearing threshold levels directly after the trauma application revealed an upper limit of threshold change that is independent of trauma bandwidth and precondition.

It is well known that directly after an acoustic overstimulation the ABR threshold lifts up and sometimes does not recover to the baseline values even several days and weeks afterward. It was usually assumed that the amount of threshold shift correlates with the severity of overstimulation-induced damages (Roberts et al., 2010; Kaltenbach, 2011). This damage in the ear then causes changes in the ascending auditory pathway (Eggermont and Roberts, 2004), including a decreased input to the brain, which might be related to the development of a chronic perception of tinnitus. Basic research on tinnitus often focuses on the duration and intensity of acoustic overstimulation (e.g., Bauer and Brozoski, 2001; Rüttiger et al., 2013; Singer et al., 2013). Both parameters play key roles for the development of tinnitus, given that the severity of the hearing impairment aggravates with increasing intensity and duration of the acoustic overstimulation. Heffner and Harrington (2002) and Suberman et al. (2011) suggest that the latter is caused by an increasing loss of hair cells in the cochlea.

Derived from the pattern of changes in ABR threshold, hearing loss can be temporary or permanent. A possibly underlying mechanism might depend on the damages resulting from overstimulation in both, outer and IHC (Chen and Fechter, 2003). Damaged single hair bundles can merge (Kujawa, 2003), the tip-links of the hair bundles can be torn apart or the cell lines can swell (Harding and Bohne, 2004). A complete loss of hair cells can follow the loss of an entire hair bundle (e.g., Harding and Bohne, 2004; Kujawa, 2003). Moreover, neurotransmitters are extensively released during acoustic overstimulation causing a swelling of synapses on the dendrites of auditory nerve neurons (Robertson, 1983). Resulting severe damage and loss of the afferent neuron terminals after noise stimulation was described by Kujawa and Liberman (2009).

All these processes in the cochlea and the auditory nerve cells can lead to an increase of ABR threshold values. In the present study an acoustic overstimulation with an intensity of 105 dB SPL and a duration of one hour resulted in a threshold shift of about 25–30 dB at frequencies above 8 kHz regardless of the bandwidth of the overstimulating noise bands. Therefore, bandwidth seems to have no impact on the amount of threshold shift measured directly after the acoustic overstimulation. Eight weeks after the acoustic overstimulation, a difference in the threshold values became obvious. Animals of the 0.25-oct group showed a permanent threshold shift. In contrast, animals of the 0.5-oct group exhibited only a temporary threshold shift (TTS). This points to the fact that the trauma bandwidth indeed has an impact on the cochlea damage, which is only detectable in long-time threshold values.

Unexpectedly, we found that the maximum threshold shift directly after the acoustic overstimulation is limited. This limit was independent of the baseline values and probably associated with the number and type of damage-affected afferent nerve fibers. IHC have a multiple innovation by 10–30 afferent fibers (Bohne et al., 1982; Liberman et al., 1990; Stamataki et al., 2006). These fibers have different threshold and spontaneous rates (Kantardzhieva et al., 2013), which increase the dynamic range of the auditory periphery and it is known that noise-induced hearing loss selectively reduce the number of fibers wit high threshold and low spontaneous rates (Furman et al., 2013). We were able to con-

firm these reductions of fibers after noise trauma by an indirect method, i.e. peak analysis of the ABR waveforms at high levels (80 dB SPL). Assuming that our ABR measurements directly after the acoustic overstimulation are influenced by this fiber loss it seems that a constant amount of noise with certain SPL and the repetition of them lead to a fiber loss of the same (high spontaneous rate) population. A repetition of the noise trauma only deceased the low spontaneous fibers in the 0.5-oct group. Due to the reduced input to the auditory path, only the 0.5-oct group (with the higher tinnitus rate) exhibited an increase of later peak amplitudes. This increased central gain could be interpreted as a functional equivalent to homeostatic plasticity on this long timescale of eight weeks, which would point to tinnitus related changes (Parra and Pearlmutter, 2007). Concerning the permanent threshold shift, even when hair cells are lost and a permanent threshold shift occur only the same population of fibers will be lost.

Depending on the severity of overstimulation-induced changes in the organ of Corti, the number of auditory nerve fibers connected to the IHCs is reduced (Kujawa or Liberman, 2009). Consequently, the signal input to the central processing areas is limited as a result of pathological changes in the frequency range of the acoustic overstimulation. This loss of excitation is assumed to be associated with the removal of inhibition to the neighboring neurons at the overstimulation border frequencies, which could lead to a stronger activity in these areas (Eggermont and Roberts, 2004). The increase of the receptive area, to compensate the lost input by deafferentation, is called the *filling-in phenomenon* (e.g., Rauschecker, 1999). This would predict an overstimulation-induced hyperactivity in two brain (border) areas and a tinnitus perception at two frequencies. Our results are in line with this finding only for overstimulation with a bandwidth of 0.5 oct. However, it was shown repeatedly (Nowotny et al., 2011; Turner et al., 2012) that these processes are changing over time making the timing of the measurements critical for the characterization of the induced tinnitus perception.

Like in humans (Noreña et al., 2002; Roberts et al., 2008), tinnitus perception was found over a wide range of frequencies and was consistent with the documented hearing impairment (narrow-band noise affected animals). Repeated acoustic overstimulation is thought to increase cochlea damage and the risk of developing tinnitus (Coomber et al., 2014). We tested this by replicating the acoustic overstimulation eight weeks after the first one. The application of the first noise trauma decreased the number of low spontaneous IHC fibers in both groups. Furthermore, trauma repetition only decreases the fiber loss in the 0.5-oct group. This further decrease of input to the auditory path did not correlate with a permanent threshold shift and higher number of tinnitus-affected animals, which was found in the 0.25-oct group. Our study shows that the first noise trauma is the crucial factor for developing tinnitus and only a slight increase in the tinnitus risk can be forced by repetition of the noise trauma. An explanation for this slight increase in tinnitus-affected animals could be that the number of animals treated with broadband noise was already high after the first overstimulation. Probably because of the high number of tinnitus-affected animals after the first overstimulation, a further increase was impossible. This leads to an interesting open question for further research: Why is it that not all animals exposed to loud noise develop tinnitus afterward? Several studies on different animal models show that not all animals develop tinnitus after noise exposure (e.g., Engineer et al., 2011; Koehler and Shore, 2013; Rüttiger et al., 2013). There could be predispositions by a certain *activity state* of the brain at the time point of input loss (Ahlf et al., 2012) or the non-auditory systems, such as the limbic system, could have an influence on the development of tinnitus (e.g., Singer et al., 2013). Emotions and attention are controlled by the limbic system. Therefore, the limbic system could have an influence on the tinnitus sensation (Wallhäusser-Franke et al., 2003; Rauschecker et al., 2010). This is supported by the fact that people suffering from tinnitus perceive the phantom noise under stress conditions often louder or more intensely.

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REFERENCES

Ahlf S, Tziridis K, Korn S, Strohmeyer I, Schulze H (2012) Predisposition for and prevention of subjective tinnitus development. PLoS One 7:e44519.

Althen H, Wittekindt A, Gaese B, Kössl M, Abel C (2012) Effect of contralateral pure tone stimulation on distortion emissions suggests a frequency-specific functioning of the efferent cochlear control. J Neurophysiol 107(7):1962–1969.

Bauer CA, Brozoski TJ (2001) Assessing tinnitus and prospective tinnitus therapeutics using a psychophysical animal model. J Assoc Res Otolaryngol 2:54–64.

Bohne BA, Kenworthy A, Carr CD (1982) Density of myelinated nerve fibers in the chinchilla cochlea. J Acoust Soc Am 72:102–107.

Chen G-D, Fechter LD (2003) The relationship between noise-induced hearing loss and hair cell loss in rats. Hear Res 177:81–90.

Coomber B, Berger JI, Kowalkowski VL, Shackleton TM, Palmer AR, Wallace MN (2014) Neural changes accompanying tinnitus following unilateral acoustic trauma in the guinea pig. Eur J Neurosci 40:2427–2441.

Davis A, Rafaie EE (2000) Epidemiologie of tinnitus. In: Tyler RS, editor. Tinnitus handbook. San Diego, CA: Singular. p. 1–23.

Dehmel S, Pradhan S, Koehler S, Bledsoe S, Shore S (2012) Noise overexposure alters long-term somatosensory-auditory processing in the dorsal cochlear nucleus-possible basis for tinnitus-related hyperactivity? J Neurosci 32:1660–1671.

Eggermont JJ, Komiya H (2000) Moderate noise trauma in juvenile cats results in profound cortical topographic map changes in adulthood. Hear Res 142:89–101.

Eggermont JJ, Roberts LE (2004) The neuroscience of tinnitus. Trends Neurosci 27:676–682.

Eggermont JJ (2012) What is tinnitus? In: Eggermont JJ, editor. The neuroscience if tinnitus. Oxford University Press.

Engineer NND, Riley JRJ, Seale JDJ, Vrana WA, Shetake JA, Sudanagunta SP, Borland MS, Kilgard MP (2011) Reversing pathological neural activity using targeted plasticity. Nature 18:101–104.

Furman AC, Kujawa SG, Liberman MC (2013) Noise-induced cochlear neuropathy is selective for fibers with low spontaneous rates. J Neurophysiol 110:577–586.
Gaese BH, Nowotny M, Pilz PKD (2009) Acoustic startle and prepulse inhibition in the Mongolian gerbil. Physiol Behav 98:460–466.
Goss-Sampson MA, Kriss A (1991) Effects of pentobarbital and ketamine-xylazine anaesthesia on somatosensory, brainstem auditory and peripheral sensory-motor responses in the rat. Lab Anim 25:360–366.
Harding GW, Bohne BA (2004) Temporary DPOAE level shifts, ABR threshold shifts and histopathological damage following below-critical-level noise exposures. Hear Res 196(1–2):94–108.
Heffner H, Harrington I (2002) Tinnitus in hamsters following exposure to intense sound. Hear Res 170:83–95.
Hoffman HS, Ison JR (1980) Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. Psychol Rev 87 (2):175–189.
House JW, Brackmann DE (1981) Tinnitus: surgical treatment. Ciba Found Symp 85:204–216.
Jewett DL, Romano MN, Williston JS (1970) Human auditory evoked potentials: possible brain stem components detected on the scalp. Science 167:1517–1518.
Kaltenbach JA (2011) Tinnitus: models and mechanisms. Hear Res 276:52–60.
Kantardzhieva A, Liberman MC, Sewell WF (2013) Quantitative analysis of ribbons, vesicles, and cisterns at the cat inner hair cell synapse: correlations with spontaneous rate. J Comp Neurol 521:3260–3271.
Koch M (1999) The neurobiology of startle. Prog Neurobiol 59:107–128.
Koehler SD, Shore SE (2013) Stimulus timing-dependent plasticity in dorsal cochlear nucleus is altered in tinnitus. J Neurosci 33:19647–19656.
König O, Schaette R, Kempter R, Gross M (2006) Course of hearing loss and occurrence of tinnitus. Hear Res 221:59–64.
Kraus KS, Canlon B (2012) Neuronal connectivity and interactions between the auditory and limbic systems. Effects of noise and tinnitus. Hear Res 288:34–46.
Kreuzer PM, Landgrebe M, Schecklmann M, Staudinger S, Langguth B (2012) TRI Database Study Group. Trauma-associated tinnitus: audiological, demographic and clinical characteristics. PLoS One 7(9):e45599.
Kujawa SG (2003) Noise-induced hearing loss. In: Ballenger JJ, Snow JB, editors. Ballenger's Otorhinolaryngology: Head and Neck Surgery. Shelton, CT: BC Decker. p. 265–272.
Kujawa SG, Liberman MC (2009) Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. J Neurosci 29:14077–14085.
Lenarz T, Schreiner C, Snyder RL, Ernst A (1993) Neural mechanisms of tinnitus. Oto-Rhino-Laryngology 249:441–446.
Liberman MC (1984) Single-neuron labeling and chronic cochlear pathology. I .Threshold shift and characteristic-frequency shift. Hear Res 16:33–41.
Liberman MC, Dodds LW, Pierce S (1990) Afferent and efferent innervation of the cat cochlea: quantitative analysis with light and electron microscopy. J Comp Neurol 301:443–460.
Lin HW, Furman AC, Kujawa SG, Liberman MC (2011) Primary neural degeneration in the Guinea pig cochlea after reversible noise-induced threshold shift. J Assoc Res Otolaryngol 12:605–616.
Lobarinas E, Hayes SH, Allman BL (2013) The gap-startle paradigm for tinnitus screening in animal models: limitations and optimization. Hear Res 295:150–160.
Lockwood AH, Wack DS, Burkard RF, Coad ML, Reyes SA, Arnold SA, Salvi RJ (2001) The functional anatomy of gaze-evoked tinnitus and sustained lateral gaze. Neurology 56:472–480.
Longenecker RJ, Galazyuk AV (2011) Development of tinnitus in CBA/CaJ mice following sound exposure. J Assoc Res Otolaryngol 12:647–658.
Manzoor NF, Gao Y, Licari F, Kaltenbach JA (2013) Comparison and contrast of noise-induced hyperactivity in the dorsal cochlear nucleus and inferior colliculus. Hear Res 295:114–123.
Melcher JR, Sigalovsky IS, Guinan JJ, Levine RA (2000) Lateralized tinnitus studied with functional magnetic resonance imaging: abnormal inferior colliculus activation. J Neurophysiol 83:1058–1072.
Nicolas-Puel C, Akbaraly T, Lloyd R, Berr C, Uziel A, Rebillard G, Puel JL (2006) Characteristics of tinnitus in a population of 555 patients: specificities of tinnitus induced by noise trauma. Int Tinnitus J 12(1):64–70.
Niu Y, Kumaraguru A, Wang R, Sun W (2013) Hyperexcitability of inferior colliculus neurons caused by acute noise exposure. J Neurosci Res 91:292–299.
Noreña A, Micheyl C, Chéry-Croze S, Collet L (2002) Psychoacoustic characterization of the tinnitus spectrum: implications for the underlying mechanisms of tinnitus. Audiol Neurootol 7:358–369.
Noreña AJ, Farley BJ (2013) Tinnitus-related neural activity: theories of generation, propagation, and centralization. Hear Res 295:161–171.
Nowotny M, Remus M, Kössl M, Gaese BH (2011) Characterization of the perceived sound of trauma-induced tinnitus in gerbils. J Acoust Soc Am 130:2827–2834.
Okamoto H, Stracke H, Stoll W, Pantev C (2010) Listening to tailor-made notched music reduces tinnitus loudness and tinnitus-related auditory cortex activity. Proc Natl Acad Sci U S A 107:1207–1210.
Parra LC, Pearlmutter BA (2007) Illusory percepts from auditory adaptation. J Acoust Soc Am 121:1632–1641.
Popelár J, Syka J, Berndt H (1987) Effect of noise on auditory evoked responses in awake guinea pigs. Hear Res 26:239–247.
Pujol R, Puel JL (1999) Excitotoxicity, synaptic repair, and functional recovery in the mammalian cochlea: a review of recent findings. Ann N Y Acad Sci 884:249–254.
Rauschecker JP (1999) Auditory cortical plasticity: a comparison with other sensory systems. Trends Neurosci 22:74–80.
Rauschecker JP, Leaver AM, Mühlau M (2010) Tuning out the noise: limbic–auditory interactions in tinnitus. Neuron 66:819–826.
Roberts LE, Moffat G, Baumann M, Ward LM, Bosnyak DJ (2008) Residual inhibition functions overlap tinnitus spectra and the region of auditory threshold shift. J Assoc Res Otolaryngol 9:417–435.
Roberts LE, Eggermont JJJ, Caspary DM, Shore SE, Melcher JR, Kaltenbach JA (2010) Ringing ears: the neuroscience of tinnitus. J Neurosci 30:14972–14979.
Robertson D (1983) Functional significance of dendritic swelling after loud sounds in the guinea pig cochlea. Hear Res 9:263–278.
Rüttiger L, Singer W, Panford-Walsh R, Matsumoto M, Lee SC, Zuccotti A, Zimmermann U, Jaumann M, Rohbock K, Xiong H, Knipper M (2013) The reduced cochlear output and the failure to adapt the central auditory response causes tinnitus in noise exposed rats. PLoS One 8:e57247.
Schaette R, Kempter R (2006) Development of tinnitus-related neuronal hyperactivity through homeostatic plasticity after hearing loss: a computational model. Eur J Neurosci 23: 3124–3138.
Scholl B, Wehr M (2008) Disruption of balanced cortical excitation and inhibition by acoustic trauma. J Neurophysiol 100:646–656.
Serra L, Novanta G, Sampaio AL, Augusto Oliveira C, Granjeiro R, Braga SC (2015) The study of otoacoustic emissions and the suppression of otoacoustic emissions in subjects with tinnitus and normal hearing: an insight to tinnitus etiology. Int Arch Otorhinolaryngol 19:171–175.
Singer W, Zuccotti A, Jaumann M, Lee SC, Panford-Walsh R, Xiong H, Zimmermann U, Franz C, Geisler H-S, Köpschall I, Rohbock K, Varakina K, Verpoorten S, Reinbothe T, Schimmang T, Rüttiger L, Knipper M (2013) Noise-induced inner hair cell ribbon loss disturbs central arc mobilization: a novel molecular paradigm for understanding tinnitus. Mol Neurobiol 47:261–279.

Smith DI, Mills JH (1989) Anesthesia effects: auditory brain-stem response. Electroencephalogr Clin Neurophysiol 72:422–428.
Spoendlin H (1971) Primary structural changes in the organ of Corti after acoustic overstimulation. Acta Otolaryngol 71:166–176.
Spoendlin H, Schrott A (1987) The block surface method for evaluation of human inner ears. Acta Otolaryngol Suppl 436:25–36.
Stamataki S, Francis HW, Lehar M, May BJ, Ryugo DK (2006) Synaptic alterations at inner hair cells precede spiral ganglion cell loss in aging C57BL/6J mice. Hear Res 221:104–118.
Suberman TA, Campbell AP, Adunka OF, Buchman CA, Roche JP, Fitzpatrick DC (2011) A gerbil model of sloping sensorineural hearing loss. Otol Neurotol 32:544–552.
Syka J, Rybalko N, Popelář J (1994) Enhancement of the auditory cortex evoked responses in awake guinea pigs after noise exposure. Hear Res 78:158–168.
Turner J, Larsen D, Hughes L, Moechars D, Shore S (2012) Time course of tinnitus development following noise exposure in mice. J Neurosci Res 90:1480–1488.
Valentine PA, Teskey GC, Eggermont JJ (2004) Kindling changes burst firing, neural synchrony and tonotopic organization of cat primary auditory cortex. Cereb Cortex 14:827–839.
Wallhäusser-Franke E, Mahlke C, Oliva R, Braun S, Wenz G, Langner G (2003) Expression of c-fos in auditory and non-auditory brain regions of the gerbil after manipulations that induce tinnitus. Exp Brain Res 153:649–654.
Wang J, Ding D, Salvi RJ (2002) Functional reorganization in chinchilla inferior colliculus associated with chronic and acute cochlear damage. Hear Res 168:238–249.
Wigand ME, Hellweg FC, Berg M (1982) Tinnitus after microsurgical interventions at the internal auditory canal (author's transl). Laryngol Rhinol Otol (Stuttg) 61:132–134.
Yang G, Lobarinas E, Zhang L, Turner J, Stolzberg D, Salvi R, Sun W (2007) Salicylate induced tinnitus: behavioral measures and neural activity in auditory cortex of awake rats. Hear Res 226:244–253.
Zeng F (2013) An active loudness model suggesting tinnitus as increased central noise and hyperacusis as increased nonlinear gain. Hear Res 295:172–179.

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